THE PRIOR ART DOES NOT ANTICIPATE APPLICANTS INVENTION UNDER 35 U.S.C. 102(A)

The Examiner rejected claim 38 as being anticipated by Corish *et al.* (Protein Engineering 12 (12) 1035-1040, 1999). The Examiner argued that the coupling of a PEST domain to the C-terminal end of GFP and a cyclin B1 destruction box to the N-terminus of the protein is in essence coupling two copies of a destabilization domain (i.e. a linear multimerized destabilization domain) that is non-cleavable by alpha-NH-ubiquitin protein endoproteases to a target protein.

To be anticipatory under 35 U.S.C. §102, a reference must teach each and every element of the claimed invention. See Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F. 2d 1367, 1379 (Fed. Cir. 1986). "Invalidity for anticipation requires that all elements and limitations of the claim are found within a single prior art reference. ...There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." See Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565 (Fed. Cir. 1991).

In contrast to the method disclosed in Corish *et al.*, the claim at issue recites the use of a " linear multimerized destabilization domain," in order to destabilize the protein of interest. This term, which is defined in the specification on page 15, line 16: refers to a multimerized destabilization domain as comprising at least two destabilization domains that are linearly coupled together. This differs from Corish *et al.*, where the destabilization domains are not coupled together, but attached to either terminus of the protein of interest.

Because Corish *et al.* does not teach a linear multimerized destabilization domain, it cannot anticipate claim 38, and Applicants accordingly request withdrawal of this rejection of the claim.

THE PRIOR ART FAILS TO MAKE APPLICANTS' INVENTION OBVIOUS UNDER 35 U.S.C. § 103(A)

The Examiner rejected claims 38 to 40 as being obvious under 35 USC §103(a) in light of Johnson *et al.* (EMBO 11 (2) 497-505, 1992) in view of Hochstrasser (Ann. Rev. Genet. 30; 405-439, 1996).

The Examiner argued that Johnson *et al.* discloses a ubiquitin domain containing an amino acid substitution at position 76, that is non-cleavable by alpha NH –ubiquitin protein endoproteases, and that results in the rapid degradation of the target protein when fused to the N-terminus of the protein. The Examiner further stated that Johnson *et al.* does not teach multimerization of the linear destabilization domain.

The Examiner argued that Hochstrasser teaches that degradation of some proteins is accelerated by multiubiquination *in vitro*, and therefore that it would be obvious to combine the teachings of Johnson *et al.* and Hochstrasser to couple a target protein to a linear multimerized, and non-cleavable destabilization domain. Further the Examiner argued that a person of ordinary skill in the art, based on the teachings of the prior art, would have a reasonable expectation of success that such a construct would have an accelerated rate of degradation.

Applicants respectfully disagree with the Examiners conclusions, and request reconsideration of the Examiners rejection.

To establish a prima facie case of obviousness a three-prong test must be met. First, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. Second, there must be

a reasonable expectation of success found in the prior art. Third, the prior art referenced must teach or suggest all the claim limitations. In re Vaeck, 947 F. 2d 488 (Fed. Cir. 1991).

As concluded by the Examiner Johnson *et al.* neither discloses nor suggests the use of a linear multimerized destabilization domain in order to accelerate the degradation of a target protein.

By contrast, Johnson *et al.*, discloses that the creation of a single, non-cleavable ubiquitin fusion protein already results in the rapid degradation (t ½ = to 5 minutes) of the target protein. Because the half-life reported for this construct was the shortest of all the constructs tested, the data in Johnson *et al.*, directly supports the proposition that the rate of degradation of this construct is already maximal, and that further addition of destabilization domains would not be likely to further increase the rate of degradation.

Accordingly Johnson *et al.*, fails to provide any motivation or create an expectation of success, that the creation of fusion proteins comprising multiple non-cleavable ubiquitin domains, as disclosed in the present application, would accelerate protein degradation.
By contrast Johnson *et al.* points to the non-obviousness of the claimed invention.

Hochstrasser discloses that *in vitro*, multi-ubiquitination of proteins via the attachment of poly-ubiquitin chains, (in which the ubiquitin monomers are coupled together via isopeptide bonds attached to the α-amino group of lysine), accelerates the degradation of some proteins.

Hochstrasser neither discloses nor suggests a linear multimerized destabilization domain that is non-cleavable by a α-NH ubiquitin protein endoprotease.

Unlike the present invention, the multi-ubiquitin monomers, disclosed in Hochstrasser are typically attached post-translationally as branched chains, connected via lysine residues throughout the target protein.

Because the multi-ubiquitin chains disclosed in Hochstrasser are attached in a different way, and at multiple different positions on the target protein, a person of ordinary skill in the art could have no expectation of success that the attachment of a linear multimerized destabilization domain, as described in the present application, would function as naturally occurring, multi-branched poly ubiquitin chains to accelerate protein degradation.

Furthermore Hochstrasser teaches away from the Applicants claimed invention by suggesting that ubiquitin attachment could directly trigger protein unfolding (page 418, lines 17 to 20), and that the attachment of ubiquitin chains at different sites in a protein could result in alternative metabolic fates of that protein (page 418, last paragraph).

Accordingly, Hochstrasse fails to provide any motivation, or create an expectation of success that the creation of fusion proteins comprising multiple non-cleavable ubiquitin domains, as disclosed in the present application, would accelerate protein degradation.

The Supreme Court held in U.S. v. Adams, 383 US 39, 148 USPQ 479 (1996) that such teaching away is an important indicator of non-obviousness. Specifically the Federal Circuit has found that teaching away from the art is a *per se* demonstration of lack of prima facie obviousness. *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ 2d 1529 (Fed. Cirri. 1988); *In re Fine*, 837 F. 2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Nielson*, 816 F.2d 1567, 2 USPQ2d 1525 (Fed. Cir. 1987).

Thus the scope and content of the prior art, when taken in its entirety, and including passages that teach away from the claimed invention, is significantly different from the currently pending claims at issue. The combination of references cited by the Examiner neither provides any motivation nor suggestion to modify the primary reference. The prior art fails to provide a reasonable expectation of success that modification of the primary reference, to create the claimed invention, would be successful. Finally the combination of the references, resulting in the addition of multiple branch chained non-cleavable ubiquitin monomers to a protein of interest, would not result in the claimed invention.

Accordingly, Applicants respectfully submit that the Examiner withdraw this rejection of the claims.

The Examiner rejected claim 50 as being obvious under 35 USC §103(a) in light of Hay *et al.* (WO 99/47640) in view of Johnson *et al.* (EMBO 11 (2) 497-505, 1992) and further in view of Hochstrasser (Ann. Rev. Genet. 30; 405-439, 1996).

The Examiner argued that Hay *et al.* discloses a reporter polypeptide coupled to a repressor polypeptide via a polypeptide linker containing a protease site. Cleavage of the linker results in an increase in activity of the reporter polypeptide. The Examiner stated that Hay *et al.* does not teach that the repressor polypeptide can be a multimerized, non-cleavable, destabilization domain. However, the Examiner concluded that it would have been obvious to combine the teachings of Hay *et al.* and Hochstrasser to construct a molecule that substitutes the repressor protein of Hay *et al.* with a linear multimerized destabilization domain that is non-cleavable by an α-NH ubiquitin protein endoprotease. Further the Examiner argued that it would also have been obvious to construct the linker moiety such that it was also non-cleavable by a α-NH ubiquitin protein endoprotease.

Applicants respectfully traverse the Examiners' rejection and request reconsideration.

As concluded by the Examiner, Hay *et al.* neither discloses nor suggests the use of a linear multimerized destabilization domain in order to accelerate the degradation of a target protein.

Unlike the present invention, Hay *et al.* describes the repressor protein (See, page 14, lines 7 to 11 of Hay *et al.*) as: "...a polypeptide that is capable of inhibiting or reducing the activity or inhibiting the ability to detect a reporter polypeptide ***to which it is bound***. The repressor may interact directly with the reporter polypeptide to repress its activity or function. Alternatively, the repressor ***may confer a specific localization in the cell*** such that the reporter has reduced activity. In one embodiment, the repressor is a transmembrane polypeptide." (Emphasis added)

Thus the function, and nature of the repressor protein disclosed in Hay *et al.*, and the multimerized destabilization domains disclosed in the present invention, are completely different.

Accordingly Hay *et al.*, fails to provide any suggestion, motivation or create an expectation of success, that multiple non-cleavable ubiquitin domains, as disclosed in the present application, could be substituted for the repressor polypeptide disclosed in Hay *et al.* Furthermore, while the linker in Hay *et al.* may inherently lack a α-NH ubiquitin protein endoprotease site, there is no teaching or suggestion that a person of ordinary skill in the art should construct the linker moiety to lack such a feature. In fact this limitation is novel and non obvious over the prior art of record.

Furthermore neither Johnson *et al.* nor Hochstrasser alone, or in combination with Hays *et al.* teach or suggest the construction of a molecule that substitutes the repressor protein of Hay *et al.* with a linear multimerized destabilization domain that is non-cleavable by an α-NH ubiquitin protein endoprotease.

Additionally as discussed above, neither Johnson *et al.* nor Hochstrasser either alone, or in combination, disclose or suggest the use of a linear multimerized destabilization domain, as disclosed and claimed in the present specification.

Thus the combination of references cited by the Examiner neither provides any motivation nor suggestion to modify the primary reference. The prior art fails to provide a reasonable expectation of success that modification of the primary reference, to create the claimed invention, would be successful. Finally the combination of the references, does not teach all the elements of claim 50. Accordingly the combination of these references cannot make the claimed invention obvious.

For this reason, Applicants respectfully request withdrawal of the rejection of claim 50 under 35 U.S.C. §102(a).

REJECTION OF CLAIMS UNDER 35 U.S.C. § 112 FIRST PARAGRAPH

The Examiner rejected claims 1 to 15, 19 to 28, 31 to 37 and 60 under 35 U.S.C. §112 first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with the claims.

Specifically the Examiner is of the opinion that although the specification discloses how the claimed methods can be used in vitro, the state of the art of the production of transgenic animals at the time of filing was unpredictable. The Examiner cited references to show the potential difficulties of producing animals of different species with identical phenotypes, the variable expression, or lack of expression of transgenes, and the variation in phenotypes observed in animals that express the same transgene.

The Examiner concluded that phenotype of a theoretical transgenic animal was unpredictable, and that therefore one of skill in the art could not readily predict that any non-human transgenic animal would have the desired phenotype required to be useful for the methods claimed.

Applicants respectfully traverse the rejection and request reconsideration.

The absence of undue experimentation is the standard for enablement (In *re Wands*, 8 USPQ2d 1400). In other words, the application must teach the skilled artisan how to make and use the claimed invention with no more than routine or 'undue' experimentation (In *re Wands*).

It is not required that the applicant provides a working exemplification for every embodiment of a claim, nor spell out every detail. See MPEP 608.01(h) ("A patent specification is not intended nor required to be a production specification"); see also in *re Marzochi and Horton* (169 USPQ 367) (" The first paragraph of §112 requires nothing more than objective enablement. How such a teaching is set forth, either by use of illustrative examples or by broad terminology, is of no importance.")

The claims at issue are directed to host cells, or methods of detecting, or regulating, the concentration of one or more proteins in a cell via a multimerized destabilization domain, that is non-cleavable by an α-NH ubiquitin protein endoprotease.

Although the production of particular transgenic animals with specific phenotypes may be to a certain extent unpredictable, ***the claims at issue*** are not directed to methods of producing adult transgenic animals exhibiting a specific phenotype.

By contrast, the method claims are directed to methods of detecting activity in a cell, or regulating the concentration of a target protein in a cell, or the host cell itself. These methods are fully enabled to the full scope of the claim by the specification as originally filed.

Specifically the specification describes the use of a multimerized destabilization domain, containing variable numbers of destabilization domains. Surprisingly the present inventors have discovered that increasing the number of these destabilization domains results in a reproducible and predictable increase in relative destabilization. The approach has the advantage that the degree of destabilization can be accurately controlled by varying the number of destabilization domains added to the target protein to take into account the various factors that typically result in variable levels of protein expression.

In the present invention, the desired level of protein expression can be obtained by simply conducting routine experimentation to find the optimal construct for the specific cellular background. For example, by transfecting into separate cells constructs containing, 1, 2, 3, 4 or 5 copies of the destabilization domain, and by comparing the expression of the protein of interest between the cells, cells exhibiting the appropriate level of expression of the protein of interest can be readily determined. For example, by a variety of standard approaches, such as western blotting.

Applicants point out that the references and arguments cited by the Examiner are directed towards the difficulties of reproducibly creating phenotypes in different adult transgenic organisms. Many of these difficulties are not directly related to the control of protein expression within the cell, as in the current claims, and are therefore irrelevant to the claims at issue.

It is well settled that the disclosure of the invention set forth by Applicants in their application must be given the presumption of correctness and operativeness by the PTO. The only relevant

concern of the PTO under the circumstances should concern the truth of the assertions contained in the application. In re Marzocchi, 439 F. 2d 220, 169 U.S.P.Q. 367 (C.C.P.A. 1967); see also In re Bowen, 492 F. 2d 859, 181 U.S.P.Q. 48 (C.C.P.A. 1974).

The Examiner has so far failed to provide any evidence to controvert the truth of Applicants' assertions in the instant specification.

For all the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1 to 15, 19 to 28, 31 to 37 and 60 under 35 U.S.C. §112 first paragraph.

REJECTION OF CLAIMS UNDER 35 U.S.C. § 112 SECOND PARAGRAPH

The Examiner has rejected claims 22 to 28 and 31 to 37 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicant regards as the invention.

Specifically the Examiner rejected claim 22 because the claim contains the limitation "said test chemical" which lacks antecedent basis. Applicants' claims as originally filed fully met the requirements of 35 U.S.C. 112, second paragraph. To expedite the allowance of the currently pending claims, however, Applicants have amended claim 22 by changing its dependency such that it is now dependent on claim 21. Applicants believe that this amendment fully addresses the Examiners rejection, and accordingly request the withdrawal of the rejection to claim 22, and claims 23 to 28 and 31 to 37 that depend from claim 22.

The Examiner has also rejected claim 23 because the preamble recites "a method of regulating the concentration of one or more target proteins in a cell." Specifically the Examiner is of the opinion that the claimed method discloses only how to increase the concentration of the target

protein, and does not disclose how to decrease the concentration of the target protein. Applicants respectfully traverse the rejection and request reconsideration.

The claims at issue relate to the use of the linear multimerized destabilization domain as a means of coordinately regulating the concentration of a number of target proteins in a cell simultaneously. This embodiment of the invention is specifically disclosed on pages 45 and 46 of the instant specification, a portion of which is reproduced below.

> In one embodiment the linker may contain a non-naturally occurring protease cleavage site (in that cell type), such that cleavage of the linker by the protease results in uncoupling of the target protein from the multimerized destabilization domain hence creating an increase in the stability and concentration of the target protein after protease digestion. ***In one aspect of this method, regulation of the activity of the protease can be achieved via regulating the concentration and exposure of the cell to an inhibitor of the protease.*** (See, page 46, lines 3 to 9, emphasis added).

In contrast to the Examiner's assertion, the method does enable both an increase and decrease in the concentration of the target protein from any given set level. For example, an increase in stability and hence concentration of the protein of interest may be exerted by increasing the expression, or activity, of the protease that is responsible for cleaving the linker coupling the protein of interest to the multimerized destabilization domain. Conversely, a decrease in stability and hence concentration of the protein of interest may be exerted by decreasing the expression or activity of the protease, for example by adding a protease inhibitor.

Accordingly the claimed method can be used to increase and decrease the concentration of a protein in a cell. Applicants thus request withdrawal of this claim under 35 U.S.C. §112 second paragraph.

CONCLUSION

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. Please apply any charges not covered, or any credits, to Deposit Account 50-1355.

Respectfully submitted,

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APPENDIX A – Claims amended in the present response marked to show amendments made.

22. (Amended) The method of claim [20] 21, wherein said method further comprises the step of relating said reporter moiety activity before addition of said test chemical to said reporter moiety activity after addition of said test chemical.

APPENDIX B – Currently pending claims after entry of the present amendment

1. A method of detecting an activity in a cell, comprising;

1) providing a cell comprising,

a) at least one destabilization domain, wherein said destabilization domain is non-cleavable by α-NH-ubiquitin protein endoproteases,

b) a reporter moiety, and

c) a linker moiety that operatively couples said destabilization domain to said reporter moiety,

wherein said linker moiety comprises a recognition motif for said activity and modification of said linker moiety by said activity modulates the coupling of said destabilization domain to said reporter moiety thereby modulating the stability of said reporter moiety, and

wherein said linker moiety is non-cleavable by said α-NH-ubiquitin protein endoproteases,

2) detecting said reporter moiety, or a product of said reporter moiety.

2. The method of claim 1, wherein said at least one destabilization domain is arranged as linear multimer, and

wherein said linear multimer comprises at least two copies of said destabilization domain and is non-cleavable by said α-NH-ubiquitin protein endoproteases.

3. The method of claim 1, wherein said linker moiety is non-naturally occurring polypeptide or protein.

4. The method of claim 1, wherein said linker moiety covalently couples said destabilization domain to said reporter protein.

5. The method of claim 1, wherein said linker moiety is between about 1 and 30 amino acid residues.

6. The method of claim 1, wherein said destabilization domain comprises a ubiquitin homolog.

7. The method of claim 6, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by said α-NH-ubiquitin protein endoproteases.

8. The method of claim 6, wherein said ubiquitin homolog comprises a mutation at glycine 76.

9. The method of claim 1, wherein said linker moiety comprises a first amino acid sequence that is covalently coupled to said reporter moiety, and a second amino acid sequence that is covalently coupled to said at least one destabilization domain.

10. The method of claim 1, wherein said activity is selected from the group consisting of a protease activity, a protein kinase activity and a phosphoprotein phosphatase activity.

11. The method of claim 1, wherein said reporter moiety is selected from the group consisting of a naturally fluorescent protein homolog, a β-lactamase homolog, a α-galactosidase homolog, an alkaline phosphatase homolog, a CAT homolog, and a luciferase homolog.

12. The method of claim 11, wherein said reporter moiety comprises a β-lactamase homolog.

13. The method of claim 11, wherein said reporter moiety comprises an *Aequorea* Green fluorescent protein homolog.

14. The method of claim 11, wherein said reporter moiety comprises an Anthozoan Green fluorescent protein homolog.

15. The method of claim 1, wherein said cell is a mammalian cell.

16. The method of claim 1, wherein said cell is a yeast cell.

17. The method of claim 1, wherein said cell is an insect cell.

18. The method of claim 1, wherein said cell is a plant cell.

19. The method of claim 1, wherein said method further comprises the step of adding a protein synthesis inhibitor to said cell.

20. The method of claim 1, wherein said method further comprises the step of adding an inhibitor of said reporter moiety to said cell.

21. The method of claim 1, wherein said method further comprises the step of adding a test chemical to said cell.

22. (Amended) The method of claim 21, wherein said method further comprises the step of relating said reporter moiety activity before addition of said test chemical to said reporter moiety activity after addition of said test chemical.

23. A method of regulating the concentration of one or more target proteins in a cell, comprising;
    1) providing a cell comprising,

a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain,

b) a target protein, and

c) a linker that operatively couples said linear multimerized destabilization domain to said target protein,

wherein said linker comprises a protease cleavage site for a protease and cleavage of said linker by said protease modulates the coupling of said linear multimerized destabilization domain to said target protein, thereby modulating the stability of said target protein in said cell, and

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases,

2) providing said protease to cause cleavage of said linker thereby increasing the stability and concentration of said protein of interest in said cell.

24. The method of claim 23, wherein said protease is naturally expressed in said cell.

25. The method of claim 23, wherein said protease is not naturally expressed in said cell.

26. The method of claim 23, further comprising the step of adding an inhibitor of said protease.

27. The method of claim 23, wherein said linker is between 1 and 30 amino acid residues.

28. The method of claim 23, wherein said cell is a mammalian cell.

29. The method of claim 23, wherein said cell is a yeast cell.

30. The method of claim 23, wherein said cell is an insect cell.

31. The method of claim 23, wherein said destabilization domain comprises a ubiquitin homolog.

32. The method of claim 31, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

33. The method of claim 31, wherein said ubiquitin homolog comprises a mutation at glycine 76.

34. The method of claim 23, wherein said protease is provided by transfecting said cell with an expression vector comprising a nucleic acid sequence encoding said protease.

35. The method of claim 34, wherein said expression vector further comprises an inducible promoter.

36. The method of claim 34, wherein said expression vector is a retroviral expression vector.

37. The method of claim 34, wherein said protease is a viral protease.

38. A method of destabilizing a target protein in a cell, comprising;

operatively coupling a target protein to a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain.

39. The method of claim 38, wherein said destabilization domain comprises a ubiquitin homolog.

40. The method of claim 39, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

50. A recombinant DNA molecule, comprising a nucleic acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

55. A recombinant protein molecule, comprising an amino acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

60. A host cell, comprising a nucleic acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a -NH-ubiquitin protein endoproteases.